44865723



Reviewer: Semalulu, S , Date April 20, 2001

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**STUDY TYPE:** Combined chronic/oncogenicity [feeding,]-[Rat]; OPPTS 870.4300 [§83-5]; OECD 453.

**TEST MATERIAL (PURITY):** SZX 0722 (99.4%) [Iprovalicarb]

**SYNONYMS:** Melody

**CITATION:** L. Schladt and E. Hartmann (1998): SZX 0722 - Chronic toxicity and cancerogenicity investigations in Wistar rats (administration in the feed over 24 months). Bayer AG, Wuppertal, Germany. Report no. 27160 (February 4, 1998). MRID not available. Unpublished.

Dates of experimental work: January 1994 - February 1996.

**SPONSOR:** Bayer Corporation.

**EXECUTIVE SUMMARY:**

In a combined chronic/carcinogenicity study (MRID not available), SZX 0722 (95.8% - 98.5%) was administered to 50 rats (Bor: WISW[SPF-Cpb)/sex/dose in the diet (admixed with 1% peanut oil), at concentrations of 0, 500, 5000, and 20000 ppm (0, 26.0, 262.5, or 1109.6 and 0, 31.7, 326.3, and 1379.7 mg mg/kg bw/day in males and females respectively) for 24 months. Ten additional rats/sex/dose were treated similarly and included in the study for the 12 months interim sacrifice.

There were no treatment related mortalities in either sex. At 20000 ppm there was an increase in the incidence of vaginal bleeding among females, and the males had an increase in the incidence of cataracts, and turbidity of the vitreous body after one year of treatment. Treatment related decreases in body weight gain occurred in females at 20000 ppm Plasma cholesterol levels were increased in females at 5000 ppm and above, and alkaline phosphatase activity was significantly increased in males at 20000 ppm. At interim sacrifice, the relative liver weight (compared to body mass) of females at 20000 ppm was increased (22%), and some females at 5000 ppm (2/10) and at 20000 ppm (3/10) had slight hypertrophy of hepatocytes. At study termination, there were increases in relative liver weights of females at 5000 (9%) and 20000 ppm (19%), and increased absolute liver weights of males at 20000 ppm (22%). There was also increased incidence of hepatocellular hypertrophy in females, and bile duct hyperplasia affecting both sexes at 5000 ppm and above. Furthermore, females had slightly increased incidence of thyroid follicular cell adenomas and thyroid follicular cell carcinoma at 5000 and above, with a positive trend evident for follicular adenomas. Benign transitional cell papillomas of the urinary bladder occurred in females at 20000 ppm (4%), and mixed Muellerian tumours of the uterus occurred at 5000 ppm (2%) and at 20000 ppm (4%). The increased incidence of these tumours was considered treatment related as it exceeded historical control range, or showed a positive trend. In addition, females at 20000 ppm had a slight increase in the incidence of uterine adenocarcinomas (6/50; 12%) compared to 4% (2/50) in the control group, and clitoral gland carcinomas were observed in 4% of females (2/50) at that dose, compared to 0% in the control group. Although both these tumour types were within the historical control incidence,

they may add to the number of tumours involving the uterus (Muellerian tumours) to achieve statistical and toxicological significance. In males at 20000 ppm, osteosarcomas occurred in three animals (two in the femur, one in the lower jaw) and chondrosarcoma of the nasal cavity was observed in one animal. These tumours (chondrosarcoma and osteosarcomas) were considered treatment related as both were unusual in the rat strain and outside the historical control range for the conducting laboratory.

The LOAEL in females was 5000 ppm (326.3 mg/kg bw/day), based on increases, in plasma cholesterol levels, relative liver weights, hepatocellular hypertrophy and bile duct hyperplasia, as well occurrence of uterine Muellerian tumour and a positive trend for thyroid adenomas at that dose. The NOAEL in females was 500 ppm (31.7 mg/kg bw/day).

The LOAEL in males was 5000 ppm (262.5 mg/kg bw/day), based on histopathological changes in the liver (bile duct hyperplasia) at that dose and above. The NOAEL in males was 500 ppm, (26.0 mg/kg bw/d).

The NOAEL for carcinogenic effects was 500 ppm, (26.0 mg/kg bw/d).

Dosing was considered adequate. It exceeded the limit dose and there were observations at the highest dose of clinical signs, organ and body weight, clinical chemistry, and histological changes in the liver, as well as a slight increase in the incidence of neoplasms in a variety of tissues (bones, thyroid, urinary bladder, and uterus).

This chronic/carcinogenicity study in the rat is acceptable, and satisfies the guideline requirement for a carcinogenicity study (83-2); OECD 453 in the rat

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS:

1 **Test Material:** SZX 0722

**Description:** Technical, a white powder

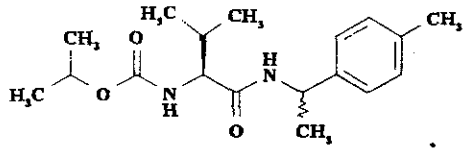
**Lot/Batch #:** NLL 4812-6.1

**Purity:** 95.8 -98.5 % a.i.

**Compound Stability:** Stable at room temperature

**CAS #:**

Structure



2. **Vehicle and/or positive control:** 1 % DAB 9 Peanut oil (Batch # 904)

3 **Test animals:**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | Hsd/WIN: WU (SPF) | |
| **Age/weight at study initiation:** | 4-5 weeks/129 g (106-152 g) males; 105 g (81-124 g) females | |
| **Source:** | Harlan-Winkelmann Animal Breeders, Brochen, Kreis Paderborn | |
| **Housing:** | Group caged by sex in Type III Macrolon cages during acclimatization, and individually caged in Type II Macrolon cages during dosing. | |
| **Diet:** | Altomin fixed formula standard diet, 1321 (Altromin, GmbH, Lage), fed *ad libitum* | |
| **Water:** | Tap water in polycarbonate bottles, provided *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 22 ± 2 °C |
| | **Humidity:** | 55 ± 5 % |
| | **Air changes:** | 15-20/hr |
| | **Photoperiod:** | 12 hrs dark/ 12 hrs artificial light |
| **Acclimation period:** | 1 week | |

## B. STUDY DESIGN:

1. **In life dates** - Start: January 1994. End: February 1996

2. **Animal Assignment/Dose Levels:** Animals were assigned to the test groups noted in Table 1, randomly using random numbers generated from the IBM Scientific Subroutine computer Package.

TABLE 1: STUDY DESIGN

| Test Group | Conc. in Diet (ppm) | Dose to animal (mg/kg bw/day) | | Main Study 24 # months | | Interim Sac. 12 # months | |
|---|---|---|---|---|---|---|---|
| | | male | female | Male | Female | Male | Female |
| Control | 0 | 0 | 0 | 50 | 50 | 10 | 10 |
| Low (LDT) | 500 | 26 | 31.7 | 50 | 50 | 10 | 10 |
| Mid (MDT) | 5000 | 262.5 | 326.3 | 50 | 50 | 10 | 10 |
| High (HDT) | 20000 | 1109.6 | 1376.7 | 50 | 50 | 10 | 10 |

3. Dose Selection: The dose levels were selected on the basis of results obtained in a sub-chronic toxicity study in Wistar rats (Schladt, L. and Watta-Gebert, and M. Rinke, 1996), which indicated reductions in body weight gain (both sexes), feed intake and food efficiency (females), and leucocytosis (males) at 20000 ppm.

4. Diet preparation and analysis:

Diets were prepared weekly, in the week preceding feeding, by mixing appropriate amounts of test substance with Altromin® 1321 standard diet (Altromin GmbH, Lage) admixed with 1% peanut oil DAB 10, to minimise dust generation, and were stored at room temperature. Homogeneity and stability were tested at on feed sample prior to feeding and thereafter approximately every 13 weeks. During the study, samples of treated food were analysed and approximately every after 11 days of storage (corresponding to the period from the day of mixing to the last day of use), for stability and concentration.

Results - **Homogeneity Analysis:** 91 - 101% (+ 2.6 - 4.2% ) expressed as of % nominal concentration.

**Stability Analysis:** 101 to 108 %, expressed as % of start concentration at day 0.

**Concentration Analysis**: 95 - 109% (± 2.3 - 4.1% ). [mean expressed as % of nominal)

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. The dosage formulations were stable in the concentration range employed and for the duration of use, and showed a homogeneous distribution as the relative standard deviation of the analytical results did not exceed 10%.

5. Statistics

Clinical chemistry, haematology, body and organ weight as well as feed and water intake were evaluated using SAS® routines. Body and organ weights were analysed using Dunnet-Test in connection with ANOVA. A Kruskal-Wallis -Test, with a Steel-Test were used to analyse feed and water intake data. All none dichotomous variables were described by sex, dose group and sampling time using the appropriate measure of central tendency (mean, median and standard deviation). For other continuously variables, other tests were used to compare test groups with controls, based on prior knowledge from previous studies.

Those tests included the Dunnett test, for comparing multiple groups with the controls, Welch test, Kruskal-Wallis test (for non parametric data) followed by Mann-Whitney-Wilcoxon test (U tests), where appropriate. Significant differences from controls were set at $p \leq 0.05$ and $p \leq 0.01$.The calculations were performed utilizing the SAS® PROC GLM program. The statistical methods used were appropriate.

## C. METHODS:

### 1. Observations:

Animals were inspected at least twice daily (once on weekends and holidays) for signs of toxicity and mortality. A detailed clinical examination of individual animals was performed at least once every week.

### 2. Body weight

Animals were weighed prior to study initiation (week 0) and thereafter, once every week up to week 13, and every two weeks from week 15 up to study termination in week 105.

### 3. Food consumption and compound intake:

Food consumption for each animal was determined, and mean daily diet consumption was calculated as grams of food/kg body weight/day. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

### 4. Ophthalmoscopic examination

Eyes were examined at the start of the study in all animals), at week 52 for controls, 5000 ppm, and 20000 ppm males, and at end of study on all surviving animals of the control and 20000 ppm dose groups.

### 5. Haematology & Clinical Chemistry:

Blood was collected in weeks 27, 53, 79 and 105, from non fasted, non-anaesthetised animals (10/sex/dose group) from the caudal veins for glucose determination, or under ether anaesthesia from the retro-orbital venous plexus for other parameters for haematology and clinical chemistry. The parameters CHECKED (X) were examined. Additional blood samples were taken at study termination for hormone determination. Samples of the liver were collected from interim and final sacrifices for determination of Phase I and Phase II enzyme activities.

## a. Haematology

| | | | |
|---|---|---|---|
| X | | X | |
| X | Hematocrit (HCT) | x | Leukocyte differential count* |
| x | Hemoglobin (HGB) | x | Mean corpuscular HGB (MCH) |
| x | Leukocyte count (WBC) | x | Mean corpusc. HGB conc.(MCHC) |
| x | Erythrocyte count (RBC) | x | Mean corpusc. volume (MCV) |
| x | Platelet count | x | Reticulocyte count |
| | Blood clotting measurements | | |
| | (Thromboplastin time) | | |
| | (Thromboplastin time) | | |
| | (Clotting time) | | |
| | (Prothrombin time) | | |

* Minimum required for carcinogenicity studies (only on Control. and HDT unless effects are observed based on Subdivision F Guidelines.

**b. Clinical Chemistry***

| | ELECTROLYTES | X | OTHER |
|---|---|---|---|
| x | Calcium | x | Albumin |
| x | Chloride | x | Blood creatinine |
| | Magnesium | | Blood urea nitrogen |
| x | Phosphates | x | Total Cholesterol |
| x | Potassium | | Globulins |
| x | Sodium | x | Glucose |
| | | x | Total bilirubin |
| | ENZYMES | x | Total serum protein (TP) |
| x | Alkaline phosphatase (ALK) | x | Triglycerides |
| | Cholinesterase (ChE) | x | Serum protein electrophores |
| | Creatine phosphokinase | | |
| | Lactic acid dehydrogenase (LDH) | | |
| x | Serum alanine amino-transferase (also SGPT) | | |
| x | Serum aspartate amino-transferase (also SGOT) | | |
| x | Gamma glutamyl transferase (GGT) | | |
| x | Glutamate dehydrogenase | | |

* Not required for carcinogenicity studies based on Subdivision F Guidelines.

**6. Urinalysis***

Urine was collected from non fasted animals in weeks 26, 52, 78 and 104. The parameters checked (X) in the table below were examined.

| | | | |
|---|---|---|---|
| X | Appearance | X | Glucose |
| x | Volume | x | Ketones |
| x | Specific gravity | x | Bilirubin |
| x | pH | x | Blood |
| x | Sediment (microscopic) | x | Nitrate |
| x | Protein | x | Urobilinogen |

* Not required for carcinogenicity studies based on Subdivision F Guidelines.

## 7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the tissues checked (x) in the table below were collected for histological examination. In addition, the organs marked (xx), were weighed.

| X | DIGESTIVE SYSTEM | X | CARDIOVASC./HEMAT. | X | NEUROLOGIC |
|---|---|---|---|---|---|
| x | Tongue | x | Aorta* | xx | Brain* |
| x | Salivary glands* | xx | Heart* | x | Peripheral .nerve* |
| x | Esophagus* | x | Bone marrow* | x | Spinal cord (3 levels)* |
| x | Stomach* | x | Lymph nodes* | x | Pituitary* |
| x | Duodenum* | xx | Spleen* | x | Eyes (optic n.)* |
| x | Jejunum* | x | Thymus* | | _____ |
| x | Ileum* | | _____ | | GLANDULAR |
| x | Cecum* | | UROGENITAL | xx | Adrenal gland* |
| x | Colon* | xx | Kidneys*+ | x | Lacrimal gland |
| x | Rectum* | x | Urinary bladder* | x | Mammary gland* |
| xx | Liver*+ | xx | Testes*+ | x | Parathyroids*++ |
| x | Gall bladder* | x | Epididymides | x | Thyroids*++ |
| x | Pancreas* | x | Prostate | | _____ |
| | _____ | x | Seminal vesicle | x | OTHER |
| | RESPIRATORY | xx | Ovaries*+ | x | Bone* |
| x | Trachea* | x | Uterus* | x | Skeletal muscle* |
| x | Lung* | | | x | Skin* |
| x | Nose | | | | All gross lesions and masses* |
| x | Pharynx | | | | |
| x | Larynx | | | | |

* Required for carcinogenicity studies based on Subdivision F Guidelines.+ Organ weight required in chronic studies.

## II. RESULTS

### A. Observations

**1. Clinical signs of toxicity -**

A high incidence of vaginal bleeding was observed in females at 20000 ppm, when compared to other dose groups (incidences: 3/4/2/10). Interestingly, a lower incidence of palpable masses (likely due to mammary tumours) occurred among the 20000 ppm females (incidences 19/21/21/4).

**2. Mortality -**

There were no treatment-related mortalities, as the cumulative mortalities were comparable to controls across the experimental groups (Table 2).

**Table 2. Cumulative mortality in animals scheduled for 2 year treatment.**

| | Males (n = 50/group) | | | | Females (n = 50/group) | | | |
|---|---|---|---|---|---|---|---|---|
| Dose | 0 | 500 | 5000 | 20000 | 0 | 500 | 5000 | 20000 |
| week | | | | | | | | |
| 1-13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-39 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 1-52 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 1-65 | 2 | 2 | 1 | 2 | 1 | 4 | 2 | 3 |
| 1-78 | 6 | 3 | 2 | 3 | 1 | 6 | 6 | 5 |
| 1-91 | 11 | 11 | 6 | 4 | 9 | 11 | 9 | 9 |
| 1-104 | 18 | 19 | 14 | 12 | 15 | 19 | 15 | 16 |
| 1-107 | 19 | 20 | 15 | 12 | 17 | 20 | 16 | 17 |

### B. Body weight

Body weight data are presented in Table 3 . Decreased body weights were noted at 5000 ppm in females (maximally 7%) and at 20000 ppm in males (maximum 8%) and females (maximum 14%) . The decrease in body weight in females at 20000 ppm was considered toxicologically significant, as it exceeded 10 %. Plots of cumutative body weight development indicate a consistent significant decrease in body weight development in females at 20000 ppm, throughout the study (Figs. 1 and 2). Body weight gain of males in the high group was slightly significantly less than the control up to week 59, after which the body weight gain of high dose males was not significantly different from the control. The body weight change in high dose males was not considered biologically significant as it was less than 10% of control weight gain, and the animals fully recovered mid way through study.

**TABLE 3: Mean body weights (BW) and body weight gains (BWG)[a]**

| Dose level | 0 (Control) | 500 | 5000 | 20000 |
|---|---|---|---|---|
| **MALES Initial BW** | 130 | 129 | 129 | 126 |
| **BWG Wk 1 (% C)** | 39 (100) | 49 (125) | 49 (125) | 44 (112) |
| **BWG Wk 1-13 (%C)** | 265(100) | 257 (96) | 257 (96) | 248 (93) |
| **BWG Wk 13-27 (% C)** | 61(100) | 58 (95) | 60 (98) | 60 (98) |
| **BWG Wk 27-51 (% C)** | 51(100) | 51 (100) | 51 (100) | 46 (90) |
| **BWG Wk 51-75 (% C)** | 20 (100) | 21 (105) | 31 (150) | 28 (140) |
| **Overall BWG Wk -1-105** | 404 | 409 | 409 | 406 |
| **FEMALES Initial BW** | 106 | 106 | 105 | 103 |
| **BWG Wk 1 (% C)** | 28 (100) | 30 (107) | 30 (107) | 29 (103) |
| **BWG Wk 1-13 (%C)** | 114 (100) | 117 (103) | 107 (94) | 103 (90)** |
| **BWG Wk 13-26 (% C)** | 26 (100) | 27 (103) | 22 (85)* | 20** (77) |
| **BWG Wk 26-52 (% C)** | 32 (100) | 37 (115) | 26 (81)* | 18* (56) |
| **BWG Wk 52-75 (% C)** | 29 (100) | 28 (97) | 23* (79) | 22** (76) |
| **Overall BWG Wk -1-104** | 220 | 225 | 205* | 190** |

[a] Data obtained from pages 46-47 in the study report. * Significantly different ($p \leq 0.05$) from the control. ** Significantly different ($p \leq 0.01$) from the control.

Figure 1: Mean body weights (grams)vs dosing period (weeks) of male rats.

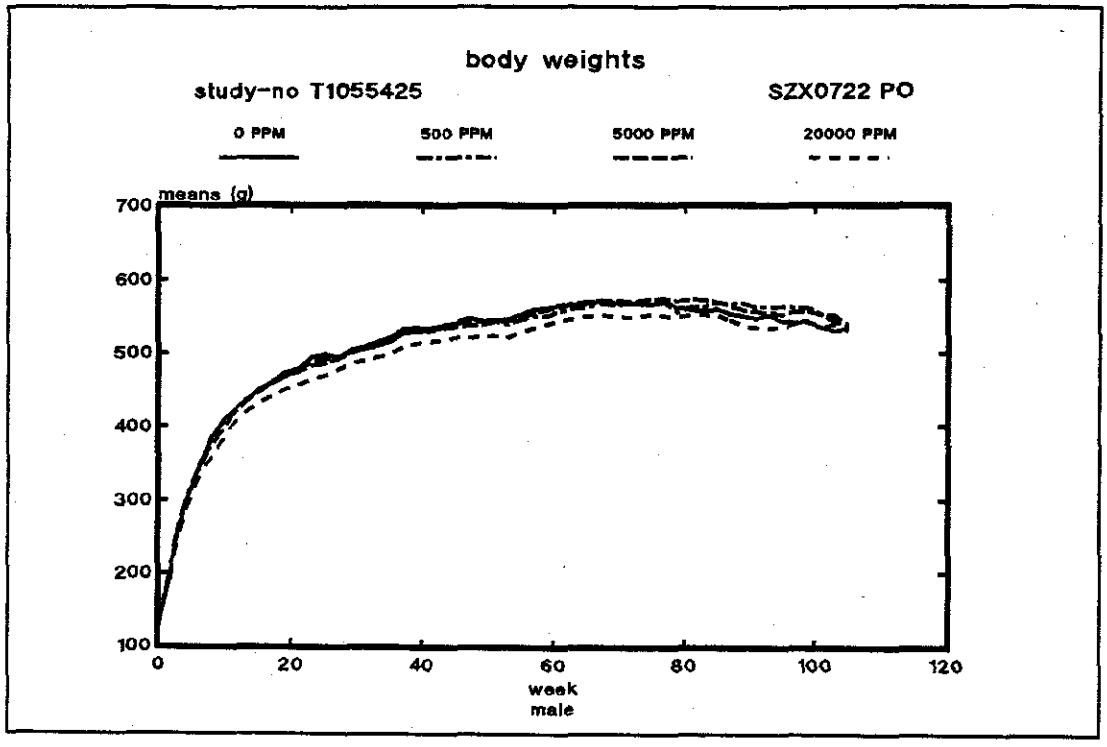
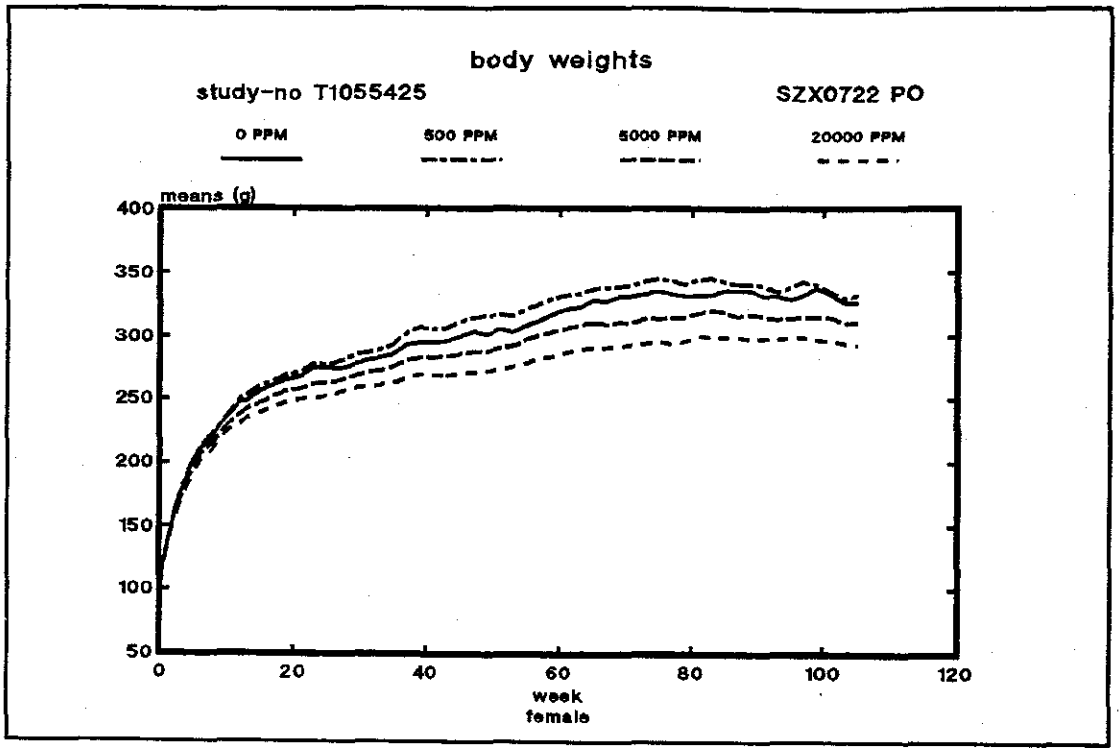


Figure 2. Mean body weight (gm) versus dosing period (weeks) for male rats



C. Food consumption and compound intake

**1. Food consumption -**

Food intake values for males and females of all dose groups did not differ significantly from control values.

**2. Compound consumption (time-weighted average)**

The calculated test compound intake has been shown in Table 1. The test compound consumed by the treated groups approximately corresponded to the theoretical differences in the dosage factor used.

**3. Food efficiency**

Food intake and and food conversion ratio (grams of food /kg body weight) is presented in Table 4. The food conversion ratio(grams food per kg of body weight gain) at 20,000 ppm was slightly decreased (94% of control) in both sexes when compared to the controls . This decrease was considered to be a toxicologically significant in female where there was a concomitant significant (≥10% ) decrease in body weight gain at the same dose

**Table 4. Food intake (g/day) and food conversion ratio (g/kg body weight)**

| Dose (ppm) | days | food consumed (g/day) | | food conversion(g/kg body weight) | |
|---|---|---|---|---|---|
| | | total | per day | total | per day |
| **Male** | | | | | |
| 0 | 736 | 16045 | 21.8 | 38319 | 52.1 |
| 500 | 736 | 16099 | 21.9 | 38258 | 52 |
| 5000 | 736 | 16271 | 22.1 | 38647 | 52.5 |
| 20000 | 736 | 16605 | 22.6 | 40832 | 55.5 |
| **Female** | | | | | |
| 0 | 736 | 12115 | 16.5 | 47263 | 64.2 |
| 500 | 736 | 12104 | 16.4 | 46701 | 63.5 |
| 5000 | 736 | 11880 | 16.1 | 48034 | 65.3 |
| 20000 | 736 | 11944 | 16.2 | 50771 | 69 |

**D. Ophthalmoscopic examination -**

After one year of treatment, the incidence of cataract, and snow ball turbidity between lens and vitreous body were higher in the 20000 ppm males compared to controls. However by study termination, the incidence of opthlmological changes in this group was comparable to controls.

**E. Blood analyses**

**1. Haematology**

The red blood parameters (erythrocyte count, proportion of reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, hematocrit, and hemoglobin concentration and erythrocyte morphology showed no significant treatment related effects in either sex at all dose levels. However, one female of the 20000 ppm dose group which had a uterine tumour associated with vaginal bleeding, had low values of erythrocyte count, proportion of reticulocytes, hemoglobin concentration and hematocrit, which had the overall tendency to lower (without statistical significance) the red blood cell parameters for that group. This was considered to be a secondary effect to tumours, rather than a hematotoxic effect. At 20000 ppm, thrombocyte counts (throughout the study) and thromboplastin time measure (at several time points) were higher than the controls in both males and females. However, the differences were small, statistical significance achieved in only 2 incidences and only a few individual values (10 out of 80) exceeded the historical control range. Therefore, the change in thrombocyte count and thromboplastin time noted in the high dose were not considered toxicologically significant.

**2. Clinical Chemistry -**

Clinical chemistry parameters which showed significant difference from control values are presented in Table 5. Activity of alkaline phosphatase (ALP) was significantly increased in males at 20000 ppm in weeks 53, 79 and 105. The change in ALP activity was considered toxicologically significant as there were associated histological changes in the liver at that dose. Activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were significantly decreased at 5000 ppm in females, and at 20000 ppm in males (only in week 53). Glutamate dehydrogenase was deceased in both sexes at 5000 ppm and above. These decreases in the activity of these enzymes was not deemed biologically significant, as tissue injury is usually associated with an increase rather than a decrease of these enzymes. Glutamyl transferase activity was not affected at any dose level. Serum cholesterol level was significantly increased in females at 5000 ppm (at week 103) and at week 20000 ppm (beginning in week 53). Total bilirubin concentration was slightly but significantly decreased in 20000 ppm females. The increased cholesterol levels were considered a toxicologically significant treatment related effect, while the decrease in bilirubin concentration was not deemed to be biologically significant.

**F. Urinalysis -**

The urine parameters of treated groups did significantly not differ from the controls in either sex at any of the dose levels.

**Table 5. Clinical chemistry values at 53 weeks and at 105 weeks**

| Dose level | | 0 ppm | | 500 ppm | | 5000 ppm | | 20000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| **Week** | | **53** | **105** | **53** | **105** | **53** | **105** | **53** | **105** |
| AST [U/l] | | 41.4 | 31.2 | 38.9 | 26.9 | 30.0 | 24.0 | 25.7* | 24.6 |
| m | | 61.6 | 61.8 | 59.9 | 52.4 | 31.9* | 29.4* | 26.7* | 30.6+* |
| | f | | | | | | | | |
| ALT [U/l] | m | 44.5 | 32.2 | 34.7 | 28.6 | 33.4 | 26.4* | 29.8** | 27.4 |
| | f | 52.7 | 50.1 | 62.9 | 42.4 | 40.5 | 33.4 | 39.0 | 34.8 |
| ALP [U/l] | m | 181 | 164 | 178 | 176 | 214 | 191 | 224* | 235** |
| GLDH [U/l] | m | 24.1 | 16.9 | 13.4 | 12.5 | 4.1 | 4.9* | 0.5* | 3.0* |
| | f | 51.3 | 62.3 | 50.4 | 45.0 | 5.8* | 6.2* | 2.4* | 2.5* |
| CHOL [nmol/l] | f | 2.45 | 2.88 | 2.76 | 3.69 | 2.99 | 3.23 | 3.42** | 3.18 |
| BILI-t [mcmol/l] | f | 2.0 | 2.2 | 1.9 | 2.0 | 1.9 | 1.9 | 1.9 | 1.6* |

Dunnett test: * $p \leq 0.05$ ** $p < 0.01$

## G. Sacrifice and Pathology

1. **Organ weight** - Organ weight changes are presented in Table 6. At interim sacrifice, the relative organ to body weight ratio of the liver was increased (22%) in females at 20000 ppm. At terminal sacrifice, among females, there was increased (9%) absolute liver weight at 20000 ppm, and increase relative liver weight (9% ) at 5000 ppm and (19%) at 20000 ppm. There were no other significant organ weight changes in either sex at any of the other dose levels. The changes in liver weight were considered treatment related toxicologically significant effects, as they were accompanied by clinical chemistry and histological changes at those doses.

**Table 6. Changes in organ weights at interim and terminal sacrifices**

| | | 0 ppm | 500 ppm | 5000 ppm | 20000 ppm |
|---|---|---|---|---|---|
| **Liver** | | | | | |
| interim sacrifice | | | | | |
| **Rel. liver weights** [mg/100 g bw] | f | 3422 | 3142 | 3637 | 4189** |
| terminal sacrifice | | | | | |
| **Rel. liver weights** [mg/100 g bw] | f | 3612 | 3700 | 3954** | 4316** |
| **Abs. liver weights** [mg] | f | 11759 | 12295 | 12204 | 12782* |

Dunnett test: * = 5 % significance level, ** = 1 % significance level, f = females

2. Gross pathology -

At terminal necropsy, incidences of pituitary gland nodules, skin nodules as well as thickened mammary glands, were decreased in females at 20000 ppm. In males, the incidence of gross surface changes in the kidney was decreased at 20000 ppm, while the incidence of pituitary gland nodules was slightly increased at the same dose. Other dose levels and the interim sacrifices had no gross necropsy changes in either sex.

The changes observed in the skin, kidney and pituitary were not considered treatment related as they occurred in incidences that were either lower than the control, not dose related or consisted of common spontaneous lesions in the strain and age of rats used.

**3. Microscopic pathology**

**a) Non-neoplastic**

The non neoplastic changes observed in the study are summarised in Table 7. Among the interim sacrifices, some females (2/10) had enlarged (hypertrophied) hepatocytes at 5000 ppm and (3/10) at 20000 ppm. At terminal sacrifice, a significantly increased incidence of hepatocellular hypertrophy was observed in females at 5000 and 20000 ppm. Increase in the incidence of focal bile duct hyperplasia was observed in males at 5000 and 20000 ppm. These liver changes were considered toxicologically significant treatment related effects as there were associated with organ weight and clinical chemistry changes in the same dose groups . In addition, at 20,000 ppm there was treatment related increased hematopoiesis in the femoral and sternal bones marrow in females, and slightly increased incidence of Leydig cell hyperplasia. There was no evidence of treatment related occulotoxic effect at any of the dose levels.

**b) Neoplastic**

The incidence rates of neoplastic changes is presented in Table 8 . There was a dose related reduction in the number of mammary gland neoplasms (adenocarcinomas, fibroadenomas), and a slightly decreased number of adenomas of the pars distalis of the pituitary gland in females at 20000 ppm. This decrease in tumour incidences was not considered to be of biological significance. A slight increase (compared to controls) in the incidence of thyroid follicular adenoma (incidences: 0 - 0 - 1 - 2) and follicular cell carcinoma (incidences: 0 - 0 - 1 - 1) was observed in females at 5000 and 20000 ppm (with a positive trend evident for adenomas). Mixed Muellerian tumours of the uterus (one having metastasis in several organs) occurred in one female at 5000 ppm, and in 2 females at 20000 ppm. In addition, at 20000 ppm, there were squamous cell carcinoma of the clitoral glands affecting two females, transitional cell papillomas of the urinary bladder ( with a positive trend) in two females and a slightly higher than control incidence (though not statistically significant) of uterine adenocarcinomas (incidences: 2 - 3 - 3 - 6). Among males at 20,000 ppm there were osteosarcomas in three animals (two in the femur, one in the lower jaw) and chondrosarcoma of the nasal cavity in one animal.

Although the total number of tumours and frequency of benign and malignant neoplasm showed no dose related increase in either sex, and while clitoral gland adenocarcinoma and uterine adenocarcinomas were within the historical control range, the positive trend in the incidences follicular adenomas and urinary bladder papillomas, and the low but significantly different from control incidence of uterine Muellerian tumours in females, and the osteosarcomas and chondrosarcoma in males, are considered to be treatment

related.

**Table 7 Histopathological (non-neoplastic) findings in rats at interim sacrifice and at study termination**

| | 0 ppm | 500 ppm | 5000 ppm | 20000 ppm |
|---|---|---|---|---|
| **Liver** | | | | |
| interim sacrifice | | | | |
| no. of animals examined f | 10 | 10 | 10 | 10 |
| **Hepatocellular hypertrophy** f | 0 | 0 | 2 | 3 |
| terminal sacrifice (female) | | | | |
| no. of animals examined | 50 | 50 | 48 | 5 |
| hepatocellular hypertrophy | 1 t** | 2 | 8** | 20** |
| bile duct hyperplasia | 18 | 17 | 18 | 22 |
| progressive nephropathy | 41 | 37 | 38 | 28 |
| adrenocortical degeneration | 18 | 14 | 13 | 12 |
| increased hematopoiesis, femur | 3 t** | 5 | 3 | 12 |
| increased hematopoiesis, sternum | 3 t** | 5 | 3 | 9 |
| mammary gland hyperplasia | 28 | 29 | 23 | 20 |
| thyroid follicular cell hyperplasia | 2 | 0 | 1 | 3 |
| pituitary gland hyperplasia (pars distalis) | 8 | 9 | 5 | 13 |
| terminal sacrifice (male) | | | | |
| no. of animals examined | 50 | 50 | 49 | 50 |
| hepatocellular hypertrophy | 0 | 1 | 0 | 0 |
| bile duct hyperplasia | 20 t** | 24 | 29* | 31* |
| progressive nephropathy | 48 | 45 | 44 | 43 |
| adrenocortical degeneration | 3 | 1 | 2 | 0 |
| increased hematopoiesis, femur | 2 | 0 | 1 | 1 |
| increased hematopoiesis, sternum | 2 | 0 | 1 | 1 |
| mammary gland hyperplasia | 0 | 0 | 0 | 0 |
| thyroid follicular cell hyperplasia | 1 | 1 | 6 | 2 |
| Leydig cell hyperplasia | 2 t* | 2 | 4 | 6 |
| pituitary gland hyperplasia (pars distalis) | 8 | 17 | 15 | 16 |

trend-test: t** = 1% significance level; ** = 1% significance level (according to Armitage, 1955)

**Table 8. Incidence of neoplastic changes at terminal sacrifice, and the related historical control % tumour incidence**

| **Incidence of tumours** (actual number of animals with the tumour) | | | | | | | | | | Historical[x] incidence % | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | | Male (M) | | | | Female (F) | | | | M | F |
| DOSE (ppm) | | 0 | 500 | 5000 | 20000 | 0 | 500 | 5000 | 20000 | 0 | 0 |
| URINARY BLADDER | (n) No. | 50 | 50 | 49 | 50 | 50 | 49 | 48 | 50 | 50 | 50 |
| Papillomas, Transitional Cell | b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 t* | 0 | 0 |
| MAMMARY GLAND | (n) No. | 50 | 50 | 49 | 49 | 50 | 49 | 48 | 50 | 50 | 50 |
| Fibroadenoma | b | 0 | 0 | 0 | 1 | 8 | 6 | 11 | 3 | 0 | 32 |
| Adenoma | b | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 10 |
| Adenocarcinoma | m | 0 | 0 | 0 | 0 | 6t** | 3 | 2 | 0 | 2 | 22 |
| UTERUS | (n) No. | | | | | 50 | 50 | 48 | 50 | 50 | 50 |
| Adenocarcinoma | m | | | | | 2 | 3 | 3 | 6 | - | 14 |
| Mixed Muellerian Tumour | m | | | | | 0 | 0 | 1 | 2t* | - | 0 |
| PITUITARY GLAND | (n) No. | 50 | 50 | 49 | 50 | 50 | 50 | 48 | 50 | 50 | 50 |
| Adenoma / Pars Distalis | b | 11 | 6 | 10 | 12 | 20 | 19 | 20 | 14 | 24 | 54 |
| THYROID GLAND | (n) No. | 50 | 50 | 49 | 50 | 50 | 50 | 48 | 50 | 50 | 50 |
| Adenoma / Follicular Cell | b | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 2 t* | 4 | 2 |
| Carcinoma / Follicular Cell | m | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 0 |
| BONE, FEMUR / KNEE JOINT | (n) No. | 50 | 50 | 49 | 50 | 50 | 50 | 48 | 50 | 50 | 50 |
| Osteosarcomas | m | 0 | 0 | 0 | 2 t* | 0 | 0 | 0 | 0 | 0 | 0 |
| BONE OTHER SITES/Lower jaw | (n) No. | 0 | 0 | 0 | 1 | | | | | | |
| Osteosarcomas | m | | | | 1 | | | | | 0 | 0 |
| BONE ALL SITES | (n) No. | 50 | 50 | 49 | 50 | | | | | | |
| Osteosarcomas | m | | | | 3 | | | | | 0 | 0 |
| Nasal Cavity | | | | | | | | | | | |
| Chondrosarcoma | | 0 | 0 | 0 | 1 | | | | | 0 | 0 |
| CLITORAL GLANDS[#] | (n) No. | | | | | 0 | 0 | 0 | 2 | - | 2 |
| Carcinoma / Squamous Cell | m | | | | | | | | 2 | - | 2 |
| TESTES Leydig cell adenoma | b | 1 | 3 | 1 | 4 | | | | | 22 | - |

t* = $p \leq 0.05$, t** = $p \leq 0.01$ (trend-test according to Peto et al., 1980) * = $p \leq 0.05$ (one-tailed pairwise group comparison according to Peto et al., 1980) [$] = no pairwise group comparison performed. Historical[x] = Historical control tumour incidence at Bayer Ag Laboratory, in 16, two-year studies, involving 50 control animals/study, conducted between 1991 - 1998, using the same (BOR:WISW) or closely related rats strains (BOR:WISW, or Hsd Cpb:WU or Hsd/WIN:WU) having a common ancestry.

## III. DISCUSSION

The incidence of Muellerian tumours was higher than the control (2 to 4 %) . There were no documented case of Muellerian tumours in the Bayer AG Lab, and no induced Muellerian tumour has been found to date in the literature. In the RITA database (Hannover, FRG), three out of a total of 2565 female rats showed this tumour, with incidence per pertinent study varying between 0 to 2%. However, a spontaneous occurrence of this tumour was recorded for the first time in 1990 at Bayer AG Laboratory. One Muellerian tumour occurred in a low dose female of a recently completed carcinogenicity study on a different compound (Bayer AG Study No. T9059203).

The incidence of uterine carcinomas was increased at 20000 (12%). However there was no significant trend, for this tumour, and a similar incidence (14%) has been reported at Bayer AG Lab (Bayer AG study report no. 25522, 1966). This incidence was within the overall historical control range, at the Bayer AG Lab (0-16%), in the RITA data base (0-14%), and in the literature (0-16%) [Bomhard E. and Rinke M. Exp. Tox. Pathol. 46 17, 1994.

Two females in the 20000 ppm dose group (4.0%) had squamous cell carcinoma of the clitoral gland, which were identified as gross nodules at necropsy. This gland is not routinely investigated in guideline studies unless macroscopic changes are observed. Therefore, the exact incidence and significance of this finding remains difficult to ascertain. The RITA database, reports one out of 20 control animals had this tumour. At Bayer AG Lab this tumour has been reported in one female each (2% incidence), in the lower of two mid dose groups, in the mid dose group, and in the control group in three separate studies with different compounds (Bayer AG reports no 26610, 25522, and in yet to be published study no. 7059067). In Fischer rats, incidences of clitoral gland neoplasms of up to 5.8% have been reported (Boorman *et al.*, 1990).

Benign neoplasms of the urinary bladder (transitional cell papillomas) were observed in two females at 20000 ppm (4.0%) , with a statistical analysis showing a positive trend for this tumour. However, compared to control, this incidence was not statistically significant. There were no accompanying preneoplastic lesions of the urothelium in the study. Historical control data at Bayer AG Lab indicate incidences of this tumour ranging from 2.1 to 2.2%. Therefore the incidence in this study is slightly higher than the historical control range, which suggests a possible treatment related effect. A supplementary $^{32}P$-post labelling assay with urinary bladder epithelium following treatment with SZX 0722 however, did not indicate DNA adduct inducing potential of for this compound.

Malignant bone tumours (osteosarcomas) occurred in 3 males (6%) at 20000 ppm. Historical control data at Bayer AG lab and the Rita database show spontaneous incidences of this tumour of 0-2% (Bomhard and Rinke M. Exp Toxic. Pathol. 46 [17], 1994). Furthermore, one chondrosarcoma of the nasal cavity was recorded in males at 20000 ppm . There was no historical data on that tumour.

A slight increase in the incidence of follicular cell adenomas (up to 4%) and follicular cell carcinomas (2%) were noted at 5000 ppm and at 20000 ppm in females. A positive statistical trend was evident for the follicular adenomas. However preneoplastic changes (eg. hypertrophy) usually associated with neoplastic change in this tissue were not observed in this study or in other studies with this test compound, and in addition, the incidence of follicular neoplasms was within historical control range of 5-6% for the Bayer AG. Lab and the RITA data base (Bomhard and Rinke M. Exp. Toxic. Pathol. 46 (17), 1994. The thyroid adenoma were not deemed treatment related.

The incidence of Leydig cell hyperplasia was slightly increased (12%) in males at 20000 ppm, however in house data at the Bayer AG Lab show incidences of this lesion ranging from 14-50%, which puts the incidence in this study below the historical control range.

**A. Investigators' conclusions:**

Histopathological investigations indicated an upward shift in certain tumour incidence at 20,000, including uterine Muellerian tumours, clitoral gland squamous cell carcinoma, transitional cell papillomas of the urinary bladder, osteosarcomas and chondrosarcoma, none of which were seen among parallel controls, and were rarely reported in historical control data. The increased in tumour incidence was small, and slightly higher than the historical control incidence. The animals manifesting the tumours received very high doses in excess of the limit dose. The standard genotoxicity studies and special $^{32}P$- post-labelling, and the tumour initiation assays did not reveal a genotoxic potential for the test compound. It is possible that the increased tumour incidence was "high dose phenomenon" as the test compound had no associated with genotoxicity nor tumour initiating properties.

The NOAEL in females was 500 ppm, (equal to 31.7 mg/kg bw/day), based on decreased body weights, changed clinical chemistry parameters (increased cholesterol concentration, decreased total bilirubin concentration), increased relative liver weights and histopathological findings (increased incidences of hepatocellular hypertrophy) at 5000 ppm.

The NOAEL in males was 5000 ppm, (equal to 262. 5 mg/kg bw/day), based on decreased body weights, increased ALP activity, slight increase in tumour incidences at 20000 ppm.

**B. Reviewer comments:**

There is evidence in this and other studies with this compound that the liver is a target organ. Therefore the increased in incidence of bile duct hyperplasia observed in both sexes at 5000 and 20000, even though not statistically significant in males is considered a treatment related, toxicologically significant effect in both sexes. The LOAEL in females was 5000 ppm (326.3 mg/kg bw/day), based on treatment related alterations in body weights, clinical chemistry parameters (increased plasma cholesterol levels), relative liver weights increases and microscopic liver changes (hepatocellular hypertrophy and bile duct hyperplasia), a positive trend for benign thyroid tumours and uterine Muellerian tumours at that dose and above. The NOAEL in females was 500 ppm (31.7 mg/kg bw/day)

The LOAEL in males was 5000 ppm (262.5 mg/kg bw/day), based on histopathological changes in the liver (bile duct hyperplasia) at that dose and above. The NOAEL in males was 500 ppm, (equal to 26.0 mg/kg bw/day)

Repeated dosing at a high dose 20000 ppm (1300 mg/kg bw) resulted in increased (albeit slight) incidences of neoplasia, including benign tumours of the thyroid and urothelium and malignant tumours of the uterus in the females, as of bone and cartilage tumours in males. The increased incidence of uterine mixed Muellerian tumours in females, bone tumours in males, although small was considered treatment related as it was in excess of the historical control range. Although the increased incidence of mixed Mullerian and bone tumours may be related to excessive stress (i.e. a high-dose phenomenon in excess of the limit dose) to the metabolising capacity of the liver microsomal system which has been demonstrated in special metabolism studies, the high dose phonomenon can not explain the presence of a mixed Mullerian tumour at the mid dose (326.3 mg/kg bw/day). While in general the tumour incidence among females at 20000 ppm was low, a

positive trend was evident for thyroid follicular adenomas and Mullerian tumour, and there were a variety of tumours in both sexes ( transitional cell papillomas of the urinary bladder, Mullerian tumour of the uterus, osteosarcomas, and chondrosarcoma) which although few in number were either unusual for the strain of rat used or were in excess of the historical control tumour incidence. Conclusions concerning carcinogenic potential of this compound in rats have to be made in light of phamacokinetic and bioavailability studies, as well as special tumour initiation studies. However the possibility that at high dose, this compound may cause tumours can not be discounted. This has to be appropriately taken into account during risk assessment, by performing low dose extrapolation (Q* determination for this compound). Dosing was considered adequate based on observation at the highest dose selected of clinical signs, body and organ weight changes, plasma enzyme changes, and histological changes in the liver, as well as increased incidence of neoplasms in a variety of tissues.

**C. Study deficiencies:**

Historical control tumour data though referred to in study were not provided. These historical control data are required to address the significance of the rare tumours reported in this study.

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Table 4 Selected Ophthalmological Findings

| Finding | | Turbid cornea | | Cataract | | Snow ball like turbidities between lens and vitreous body | | Vascularisation of cornea | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (ppm) | N | n | % | n | % | n | % | n | % |
| MALES | | | | | | | | | |
| after 12 months | | | | | | | | | |
| 0 | 117 | 5 | 4 | 2 | 2 | 1 | 1 | 0 | 0 |
| 5000 | 113 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20000 | 120 | 5 | 4 | 9 | 8 | 8 | 7 | 2 | 2 |
| after 24 months | | | | | | | | | |
| 0 | 66 | 10 | 15 | 24 | 36 | 24 | 36 | 1 | 2 |
| 20000 | 76 | 13 | 17 | 24 | 32 | 26 | 34 | 1 | 1 |
| FEMALES | | | | | | | | | |
| after 12 months | | | | | | | | | |
| 0 | 120 | 6 | 5 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20000 | 116 | 4 | 3 | 4 | 3 | 1 | 1 | 4 | 3 |
| after 24 months | | | | | | | | | |
| 0 | 69 | 3 | 4 | 21 | 30 | 16 | 23 | 2 | 3 |
| 20000 | 68 | 4 | 6 | 12 | 18 | 10 | 15 | 0 | 0 |

N= number of eyes examined
n= number of eyes with finding

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Table 14 Organ Weights (Interim Sacrifice)

Males

| Dose | Body W. | Absolute Organ Weights | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ppm | g | Brain mg | Adrenals mg | Heart mg | Liver mg | Spleen mg | Kidneys mg | Testes mg |
| 0 | 546 | 2149 | 47 | 1734 | 19262 | 824 | 3400 | 3783 |
| 500 | 551 | 2163 | 49 | 1635 | 18071 | 916 | 3402 | 4286 + |
| 5000 | 562 | 2148 | 53 | 1824 | 20144 | 935 | 3407 | 4134 |
| 20000 | 543 | 2150 | 56 | 1679 | 20925 | 906 | 3335 | 3998 |
| | | Relative Organ Weights | | | | | | |
| ppm | g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g |
| 0 | 546 | 394 | 9 | 318 | 3526 | 150 | 622 | 694 |
| 500 | 551 | 395 | 9 | 297 | 3293 | 167 | 620 | 781 |
| 5000 | 562 | 385 | 9 | 325 | 3563 | 165 | 602 | 747 |
| 20000 | 543 | 397 | 10 | 308 | 3843 | 166 | 612 | 736 |

Females

| Dose | Body W. | Absolute Organ Weights | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ppm | g | Brain mg | Adrenals mg | Heart mg | Liver mg | Spleen mg | Kidneys mg | Ovaries mg |
| 0 | 315 | 1904 | 70 | 1123 | 10776 | 561 | 2042 | 156 |
| 500 | 329 | 1917 | 77 | 1093 | 10305 | 552 | 2011 | 163 |
| 5000 | 293 | 1944 | 64 | 1016 | 10648 | 489 | 1959 | 136 |
| 20000 | 275 + | 1930 | 59 | 1066 | 11548 | 526 | 2060 | 143 |
| | | Relative Organ Weights | | | | | | |
| ppm | g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g |
| 0 | 315 | 607 | 22 | 356 | 3422 | 178 | 649 | 49 |
| 500 | 329 | 592 | 24 | 336 | 3142 | 168 | 616 | 49 |
| 5000 | 293 | 668 | 22 | 349 | 3637 | 168 | 670 | 46 |
| 20000 | 275 + | 707 ++ | 22 | 389 | 4189 ++ | 191 | 750 ++ | 53 |

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Table 15 Organ Weights (Terminal Sacrifice)

## Males

| Dose | Body W. | Absolute Organ Weights | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Brain | Adrenals | Heart | Liver | Spleen | Kidneys | Testes |
| ppm | g | mg | mg | mg | mg | mg | mg | mg |
| 0 | 536 | 2247 | 63 | 1907 | 18486 | 1214 | 3590 | 3892 |
| 500 | 542 | 2246 | 69 | 1977 | 18653 | 1286 | 3698 | 3902 |
| 5000 | 538 | 2254 | 74 | 1852 | 18332 | 1067 | 3696 | 4123 |
| 20000 | 533 | 2232 | 63 | 1815 | 19637 | 1082 | 3388 | 4152 |
| | | Relative Organ Weights | | | | | | |
| ppm | g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g |
| 0 | 536 | 430 | 12 | 364 | 3481 | 229 | 679 | 732 |
| 500 | 542 | 418 | 13 | 368 | 3477 | 241 | 691 | 720 |
| 5000 | 538 | 426 | 14 | 351 | 3438 | 198 | 703 | 763 |
| 20000 | 533 | 426 | 12 | 343 | 3695 | 202 | 642 | 784 |

## Females

| Dose | Body W. | Absolute Organ Weights | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Brain | Adrenals | Heart | Liver | Spleen | Kidneys | Ovaries |
| ppm | g | mg | mg | mg | mg | mg | mg | mg |
| 0 | 329 | 1984 | 79 | 1483 | 11759 | 776 | 2382 | 170 |
| 500 | 334 | 2002 | 77 | 1464 | 12295 | 762 | 2519 | 165 |
| 5000 | 310 | 1983 | 75 | 1383 | 12204 | 667 | 2307 | 150 |
| 20000 | 298 ++ | 2000 | 70 | 1344 + | 12782 + | 633 ++ | 2160 + | 168 |
| | | Relative Organ Weights | | | | | | |
| ppm | g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g | mg/100g |
| 0 | 329 | 611 | 25 | 457 | 3612 | 238 | 731 | 52 |
| 500 | 334 | 607 | 23 | 444 | 3700 | 231 | 765 | 50 |
| 5000 | 310 | 647 | 25 | 451 | 3954 ++ | 216 | 749 | 49 |
| 20000 | 298 ++ | 677 ++ | 23 | 454 | 4316 ++ | 213 | 729 | 57 |